Consumption of Exogenous Bifidobacteria Does Not Alter Fecal Bifidobacteria and Breath Hydrogen Excretion in Humans1–4

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ABSTRACT The hypothesis that consumption of bifidobacteria by humans would increase colonic bifidobacteria and decrease breath hydrogen excretion was examined. A commercially available strain of bifidobacteria was tracked through the gastrointestinal tract. We determined that a 12-d feeding period of $10^{10}$ cells of exogenous bifidobacteria daily was adequate to achieve a stable number of exogenous bifidobacteria in the colon. A 12-d washout period was chosen because the exogenous bifidobacteria could no longer be detected at that time. A double-blind crossover study used both male and female subjects. The order of treatment with skim milk alone or skim milk + bifidobacteria was randomized. Breath hydrogen excretion ($\mu$mol/L) and fecal counts of total bifidobacteria [log colony forming units (CFU)/g feces] were not significantly different between males and females and were not affected by consumption of exogenous bifidobacteria. Calculations based on the numbers of exogenous bifidobacteria consumed and the fecal numbers of exogenous bifidobacteria excreted suggested that numbers of the exogenous strain increased within the gastrointestinal tract. These data suggest that it is difficult to permanently alter total colonic bifidobacteria and affect physiologic function (net hydrogen in the colon as reflected by breath hydrogen) by feeding bifidobacteria, although the percentage of the total bifidobacteria represented by the exogenous strain can be affected. J. Nutr. 128: 996–1002, 1998.

KEY WORDS: • ingested bifidobacteria • fecal bifidobacteria • breath hydrogen • humans

Bifidobacteria are among the predominant colonic flora (Simon and Gorbach 1984). Unlike the majority of colonic anaerobes that utilize the Emden-Meyerhoff (glycolytic and/or hexose monophosphate) pathway, bifidobacteria degrade glucose exclusively and characteristically via the fructose-6-phosphate pathway, and gas is not a metabolic by-product of carbohydrate fermentation (Rogosa 1974). Bifidobacteria may play an important role in maintenance of gastrointestinal health, although the mechanisms responsible for the beneficial effects associated with bifidobacteria are not clearly understood (Sanders 1993). One hypothesis for the action of bifidobacteria is competition with or inhibition of potential pathogens within the small intestine and colon (Gibson and Roberfroid 1995).

Decreases in populations of potential pathogens concurrent with increased populations of bifidobacteria have been observed, both in vitro and in vivo. In vitro, bifidobacteria have been shown to suppress the growth of pathogenic microbes including Escherichia coli and Clostridium perfringens (Gibson and Wang 1994a, Wang and Gibson 1993) and bacteroides, clostridia and coliforms (Gibson and Wang 1994b). A study of rats consuming bifidobacteria with or without fructooligosaccharide (FOS)6 in skim milk showed a significant inverse relationship between C. perfringens and bifidobacteria populations in cecal contents from the rats (Gallacher et al. 1996). Gibson et al. (1995) found a significant increase in bifidobacteria and a significant decrease in the populations of bacteroides, clostridia and fusobacteria with consumption of FOS and inulin. Terada et al. (1992) reported a significant increase in bifidobacteria and concurrent decreases in clostridia and Bacteroidaceae during administration of lactulose.

C. perfringens and E. coli are organisms that produce copious amounts of hydrogen gas (Dawes and Sutherland 1992, McKay et al. 1982, Price et al. 1988). Therefore, it is possible that the decreases in these organisms after a treatment that increases colonic bifidobacteria will result in decreased colonic hydrogen gas production. Hydrogen, carbon dioxide and methane gases are metabolites of fermentation in the colon. The hydrogen gas by-products of bacterial fermentation may be removed from...
the colon by hydrogen-metabolizing microflora or excretion in breath or flatus (Calloway 1968). Hydrogen consumption as well as production is unique to the colonic bacteria (Levitt 1969, Perman and Modler 1982); the quantity of hydrogen gas present in the colon therefore reflects the net activity of these bacterial populations. A reduction in colonic hydrogen could lead to less discomfort associated with the bloating, distention and flatulence that frequently are associated with intestinal gas production. In addition, there is a growing body of evidence suggesting that abnormalities of hydrogen metabolism may play a role in the development of colonic disease (Christl et al. 1993, Gibson et al. 1991, Roediger et al. 1993).

A small number of studies exist in which breath hydrogen has been measured after a period of dietary intervention that increased colonic bifidobacteria (Bartram et al. 1994, Gibson et al. 1995, Marteau et al. 1990). In some of these studies, nondigestible carbohydrates were provided in the dietary supplements. Given that the nondigestible carbohydrates increase breath hydrogen, it is not possible to determine the specific effect of the increased bifidobacteria populations on breath hydrogen excretion from these studies. Marteau et al. (1990) conducted a study in which both methane- and non-methane-producing subjects consumed a fermented dairy product containing \( \sim 10^{10} \) Lactobacillus acidophilus, \( 10^{10} \) B. bifidum and \( 10^{6} \) mesophilic cultures daily for a 3-wk period. Hydrogen excretion after a lactulose challenge was measured before, during and after the period of dietary intervention. The values did not change significantly, although the mean area under the curve (AUC) values were lowest during the period of bifidobacteria consumption.

In this study, we tested the hypothesis that fecal bifidobacteria will increase and breath hydrogen excretion will decrease in response to daily ingestion of exogenous bifidobacteria in healthy non-methane-producing, lactose-digesting adults. Breath excretion of hydrogen has been shown to correlate strongly \( (r = 0.9) \) with hydrogen concentration in the colonic lumen (Levitt 1969). The breath hydrogen response to a consistent dose of a nonabsorbable carbohydrate such as lactulose therefore provides an index of the net hydrogen gas produced by the colonic flora. Enumeration of bacteria in fecal samples via classical microbiological techniques provided an index of the bifidobacteria as well as total anaerobic bacterial populations in the colon because fecal flora are representative of the colonic flora (Moore et al. 1978).

**SUBJECTS AND METHODS**

**Subjects.** The initial population consisted of 13 healthy volunteers (8 women and 5 men) who had not used antibiotics or consumed yogurt or products containing bifidobacteria for a minimum of 3 mo before the study. The mean age of the participants in the study was 24.7 yr \( (\text{range} \ 18–31 \ \text{y}) \). All subjects were classified as lactose digesters on the basis of a breath hydrogen test after ingestion of 500 mL skim milk \( (25 \ \text{g} \ \text{lactose}) \), and non-methane producers on the basis of breath hydrogen gas concentrations of \( <2 \ \text{ppm} \). All subjects completed the entire study. Subjects gave informed consent and the study was approved by the Institutional Review Board Human Subjects Committee at the University of Minnesota. Table 1 contains the data for age, height, weight, body mass index (BMI) and nutrient consumption for men, women and the combined groups of subjects.

**Experimental design.** The study was a 52-d, randomized, double-blind crossover repeated measures design. It was comprised of two 12-d feeding periods, each of which was followed by a 12-d washout period, and four breath gas test days. During one of the treatment periods (the bifidobacteria feeding period), subjects consumed daily 750 mL skim milk inoculated with \( 10^{6} \) colony forming units \( (\text{CFU}) \) bifidobacteria; during the other treatment period (the control feeding period), subjects consumed daily the same amount of plain skim milk. Subjects consumed their habitual diets during the feeding and washout periods.

**Preparation and consumption of milk products.** All milk consumed by subjects during the feeding periods was skim milk purchased from a local grocery store. Milk \( (750 \ \text{mL}) \) was measured and poured into paper liter beverage containers. During the bifidobacteria feeding period, the milk was inoculated with \( 10^{10} \) cells frozen concentrate of commercially available bifidobacteria (Systems Bio-Industries, Waukesha, WI). Nothing was added to the milk consumed in the control period. All milk was stored at 4 °C until the time of consumption. Samples of the milk were periodically analyzed to confirm viability of the organisms at the desired concentration. The milk portions were prepared and distributed to subjects from 1 to 3 d before consumption. We confirmed that the bifidobacteria remained viable throughout this time at the desired concentration. Subjects were instructed to store the milk at 4 °C until the time of consumption, and to drink the milk in 2–3 servings during the day. To promote compliance with the study protocol, subjects were required to maintain records of milk consumption and return the emptied cartons to the investigators.

**Normal habitual diet composition.** Before the start of the study, each subject recorded intake of all food and fluids for three consecutive days (two week days and one weekend day). The records were used to calculate normal habitual macronutrient, energy and dietary fiber intake. Nutrient calculations were performed using the Minnesota Nutrition Data System (NDS) software, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN, Food Database version 10; Nutrient Database version 25.

**Collection of fecal samples and microbiological analysis.** Each subject provided a total of 14–20 fresh fecal samples throughout the study. All samples were collected in sterile plastic bags in a laboratory near the microbiology laboratory. The samples were processed within 15 min of defecation, thereby reducing exposure to the atmosphere. The fresh fecal samples were diluted 1:3 (wt/v) with reduced sterile peptone \( (1 \ \text{g/L}) \) and homogenized in a blender for 30–60 s under flowing nitrogen. A 1.0-mL portion of the fecal homogenate was pipetted into a bottle containing 99 mL sterile peptone, and the bottle was immediately transferred to a 37 °C anaerobic chamber (Coy Laboratory Products, Grass Lake, MI). Serial dilutions ranging from \( 10^{-3} \) to \( 10^{-9} \) were prepared, and duplicate spread plates were made with 0.1-mL aliquots. Bifidobacteria were enumerated on Reinforced Clostridial Agar (Becton Dickinson, Cockeysville, MD) plates containing selective and differential agents (Muñoz and Pares 1988). The plates were incubated anaerobically at 37 °C for 72 h. Duplicate plate values were averaged and bacterial densities were expressed as the log_{10} of the number of CFU/g wet weight of feces. Randomly selected bifidobacteria colonies from samples collected during the bifidobacteria treatment and washout period were subjected to a 16S rDNA restriction fragment length polymorphism (RFLP) analysis as previously described (Kullen et al. 1997) to monitor the presence of exogenous bifidobacteria.

### Table 1

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<th>Subject age, height, weight and body mass index (BMI), and habitual intakes of energy, macronutrient, dietary fiber and lactose</th>
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1 Data represent means ± SEM.
Collection of breath gas samples and analysis. Each subject collected breath gas samples on two occasions: the baseline test (d 0) and the day immediately following the feeding period for each treatment. The same protocol was used for both tests. After an overnight fast of about 14 h, subjects collected end alveolar breath gas samples before, 0.5 h after, and then hourly for 8 h after ingestion of a 6.6 g lactulose challenge (Constulose, Barre-National, Baltimore, MD) suspended in 240 mL tap water. Lactulose is a nondigestible carbohydrate that rapidly reaches the right colon where it is fermented. Breath samples were collected in 60-mL plastic syringes fitted with three-way stopcocks. Subjects were instructed to refrain from eating, drinking (except water or black coffee), exercising and sleeping during the test (Thompson et al. 1985). All sample syringes were stored at −20°C from the time of collection until the day of analysis when samples from both the baseline and feeding period breath tests were analyzed for each subject. Average syringe storage times were 21 d for the baseline period test samples and 8 d for the feeding period test samples. The samples were analyzed for hydrogen, methane and carbon dioxide concentration by using a Quintron Model 24 Alveo-lyzer and Model DP Microlyzer (Quintron Instruments, Milwaukee, WI). Hydrogen values were corrected for atmospheric contamination of alveolar air by normalization to a F\textsubscript{CO2} of 45 mm Hg, the partial pressure of carbon dioxide in alveolar air (Kolars et al. 1984). Subjects were instructed to consume a highly absorbable dinner (meat and rice) for the evening meal before the test to minimize the baseline breath hydrogen concentration (Anderson et al. 1981). The fasting value of breath hydrogen (time point t = 0) was subtracted from each of the breath hydrogen concentration values between h 0 and 8 of the breath test. The results were plotted as a function of time for each of the subjects, and breath hydrogen excretion during the test was quantified by calculating the area under the hydrogen response curve (AUC) between h 1 and 8 of the collection.

Statistical analysis. Breath hydrogen data and bacterial populations were analyzed with a repeated measures ANOVA to determine the existence of season and treatment order effects. Differences among the breath hydrogen AUC data were compared with a Bonferroniized paired t test (Wassertheil-Smoller 1990). Bacterial populations at each time point are expressed as means of the log_{10} values of the CFU ± SEM. Differences among means were considered significant at P < 0.05. There were no differences between values obtained for men and women.

Data handling. Data from three of the subjects were excluded from all breath hydrogen analysis; one subject produced significant quantities of methane in all lactulose breath gas tests, indicating that the subject was a methane producer. Response to lactulose is dependent on breath methane-producing status (Cloarec et al. 1990) and therefore prevents comparison between methane- and non-methane-producing individuals. Changes in methane-producing status have been reported by several investigators (Gelissen et al. 1994, Peled et al. 1987, Pitt et al. 1980). One subject failed to show more than a 0.223 μmol/L H\textsubscript{2} rise in response to the lactulose challenges, indicating that the subject was a nonresponder. Absence of H\textsubscript{2} production has been reported to exist in 2–27% of individuals tested (Bond and Levitt 1977, Gilat et al. 1978). One of the subjects had extremely variable breath hydrogen values, including a high baseline value, which precluded valid interpretation of the data (Solomons 1984). As a result of the exclusion of three of the original thirteen subjects, eight subjects completed the control feeding period first, and two subjects completed the bifidobacteria feeding period first.

Pilot study. Before conducting the study described here, a pilot experiment was done to test the viability of the milk preparation and feasibility and validity of human fecal microbiological analyses and breath gas collection and analyses. An appropriate washout period to avoid crossover effects was also determined. From this pilot study, we determined that the bifidobacteria maintained in skim milk at 4°C were viable over 4 d, making this preparation practical for use in human studies and obviating the need for subjects to pick up daily milk samples. We also determined that after 12 d without consumption of the exogenous bifidobacteria, the exogenous strain was undetectable or detectable at extremely low frequencies in the feces of the subjects.

RESULTS

Fecal bifidobacteria. The mean concentrations of fecal bifidobacteria (log_{10} CFU/g wet feces) throughout the bifidobacteria + skim milk and skim milk control periods are summarized in Figure 1. Statistical analysis of these data revealed no significant treatment effects over time. The total anaerobe concentrations in the feces collected on d 0, 12 and 24 of the treatment period were unchanged (data not shown).

Measurement of the presence of exogenous bifidobacteria in the fecal samples of each individual on d 8 of the bifidobacteria + skim milk treatment period revealed that a mean of 46.3 ± 8.1% of the total fecal bifidobacteria CFU were the exogenous strain provided in the milk (Fig. 2). The percentage of exogenous bifidobacteria in each of the 12 samples ranged from 0 to 100%. Analysis of fecal samples collected on d 0 of the bifidobacteria + skim milk treatment period showed that the exogenous strain was not present in any of the subjects before the consumption of bifidobacteria.

We compared the numbers of exogenous bifidobacteria ingested with the numbers excreted by using the total CFU of bifidobacteria found in feces and the percentage of total bifidobacteria, represented by exogenous bifidobacteria on d 8, to calculate the total number of exogenous bifidobacteria present per gram of feces for individuals. When this value was
multiplied by 30 g of feces/d (a conservative estimate), we found that the mean number of exogenous bifidobacteria recovered per fecal sample was $4.62 \times 10^{10} \pm 1.66 \times 10^{10}$ CFU. The range was $1.21 \times 10^{10}$ to $7.19 \times 10^{10}$ CFU. Thus, more exogenous bifidobacteria were found in the feces than were fed, suggesting growth of this organism in oral-fecal transit.

**Breath hydrogen.** The mean concentration (above fasting levels) of hydrogen excreted during the breath gas tests as calculated by summation of AUC between h 1 and 8 is presented in Figure 3. Figure 3A shows data for subjects who consumed the skim milk control as the first treatment, followed by bifidobacteria + skim milk; Figure 3B shows data for subjects who consumed the bifidobacteria + skim milk as the first treatment, followed by the skim milk control. Analysis of the breath hydrogen data from the 10 subjects revealed a significant order by treatment interaction that required us to modify our analysis. There was a significant difference in the baseline hydrogen AUC before the two feeding periods in the two subjects who completed the bifidobacteria feeding period first (Fig. 3B, white bars), but not in the 8 subjects who completed the skim milk feeding period first (Fig. 3A, white bars). Thus we discuss the responses of the two groups separately.

Figure 3A shows the data for pre- (white bars) and post- (black bars) treatments in the subjects consuming skim milk (Control) as the first treatment and skim milk + bifidobacteria (Bifido) as the second treatment. The pretreatment hydrogen AUC did not differ between the control and bifidobacteria consumption periods, but there was a significant difference between the hydrogen AUC at the end of the control vs. the end of the bifidobacteria period (black bars). When subjects consumed bifidobacteria + skim milk (Bifido), they excreted significantly less breath hydrogen at the end of the consumption period than when they consumed skim milk only (Control), as shown in Fig. 3A (black bars). Each of the eight subjects excreted less hydrogen after the bifidobacteria feeding compared with the control period. The mean AUC after the bifidobacteria feeding was $81.2 \pm 2.5\%$ of the response after the control feeding, with individual values ranging from 69.5 to 88.3%.

Figure 3B shows data for the two subjects who completed the bifidobacteria + skim milk period (Bifido) first, then the skim milk period (Control). As noted above, these two subjects showed an initial difference in breath hydrogen excretion between the two periods (white bars). The difference in breath hydrogen between the control and bifidobacteria periods was not significant at the end of the period of consumption (black bars). The mean AUC of these subjects after the bifidobacteria feeding was $83.4 \pm 10.7\%$ of the control period response.

**DISCUSSION**

Our hypothesis was that consumption of exogenous bifidobacteria in fluid milk would increase the numbers of colonic bifidobacteria and decrease breath hydrogen excretion (a reflection of net colonic hydrogen). Our data did not support this hypothesis, although we have several indicators that further study might be warranted.

Although many health benefits have been associated with consumption of bifidobacteria (OSullivan et al. 1992), including use as biotherapeutic agents (Elmer 1996), the specific mechanisms of their action remain unknown. One of the mechanisms proposed to account for the beneficial effects of bifidobacteria is competition with potential pathogenic gas-producing and putrefactive bacteria. Several investigators have reported increased colonic bifidobacteria populations during dietary interventions supplying exogenous bifidobacteria or bifidobacteria-promoting substances (Bouhnik et al. 1992 and 1996; Buddington et al. 1996). Additional studies have reported concurrent decreases in gas producing bacteria including C. perfringens and bacteroides (Gibson et al. 1995, Terada

**FIGURE 3** Total breath hydrogen excretion in humans consuming either skim milk or skim milk + bifidobacteria. Breath hydrogen area under the curve (AUC) between h 1 and 8 of the breath hydrogen test after a lactulose challenge. Values were calculated from the breath hydrogen response curves [pre- (d 0) and post- (d 12) daily consumption of bifidobacteria + skim milk and skim milk feeding periods (pre, white bars; post, black bars)]. Subjects were initially randomly assigned to complete either the skim milk or the bifidobacteria + skim milk periods first. A significant order × treatment interaction was found; thus, the data are presented separately on the basis of which treatment was completed first. Figure 3A represents results from subjects who completed the control feeding period first ($n = 8$), and Figure 3B represents results from subjects who completed the bifidobacteria feeding period first ($n = 2$). Data represent means ± SEM. Data were analyzed by ANOVA, followed by individual treatment mean comparisons using the Bonferroni t test. Significance is defined as $P < 0.05$. In Figure 3A, a significant difference in AUC was found at the end of the bifidobacteria + skim milk period vs. the control period (skim milk), as noted by the black bars. In Figure 3B, a significant difference was found in the prefeeding AUC (white bars), but no significant difference was found in the post-feeding AUC (black bars).

**FIGURE 2** Presence of exogenous bifidobacteria in fecal samples from humans consuming either skim milk or skim milk + bifidobacteria. Percentage of total fecal bifidobacteria that were exogenous strain of bifidobacteria. Data represent the fraction of exogenous bifidobacteria in the total bifidobacteria populations in fresh fecal samples collected on d 0 and 8 of the bifidobacteria treatment period ($n = 12$).
et al. 1992). In all of these studies, the effects of the dietary interventions appear to persist only as long as the intervention persists, suggesting that the bacteria do not colonize the gastrointestinal tract, but rather pass through in a transient manner (Bouhnik et al. 1992; Kullen et al. 1997).

In our study, feeding $10^{10}$ viable CFU bifidobacteria daily in skim milk did not significantly affect the concentration of fecal bifidobacteria. Inspection of the concentration of fecal bifidobacteria from individual subjects throughout the study suggests that subjects followed one of two response patterns. In the control period, the concentration of fecal bifidobacteria of nine of the subjects was $\sim 9 \log_{10}$ CFU/g; the remaining three subjects had numbers of bifidobacteria in the control period of $\sim 6 \log_{10}$ CFU/g. The fecal concentration of bifidobacteria of all subjects during the feeding of bifidobacteria ranged from 8 to $10 \log_{10}$ CFU/g. The low concentration in the control period and dramatic response during the consumption of bifidobacteria observed in three of the subjects suggests large interindividual differences in the adult population, greater than those that have been observed in earlier studies in our laboratory or reported by other investigators (Bartram et al. 1994, Bouhnik et al. 1992 and 1996, Ikeda et al. 1994). This may be a biological phenomenon that warrants further investigation.

No changes in total fecal anaerobes were measured during the bifidobacteria consumption period, although a shift in the relative proportion of fecal bifidobacteria over time was found. The difference in the relative proportion of fecal bifidobacteria to total anaerobes was significant between the measurement at the end of the period of consumption of bifidobacteria and after 12 d of washout. Fecal bifidobacteria represented nearly 21% of the total fecal anaerobes during the treatment and fell to slightly $<5\%$ after 12 d without consumption of exogenous bifidobacteria; however, there was no statistical difference in the relative proportion of bifidobacteria to total anaerobes at the initiation of consumption and at the peak of consumption. These results compare favorably with the findings of others. Bouhnik et al. (1996) found that during administration of fermented milk containing Bifidobacterium sp., total anaerobes did not change, whereas a significant increase in total fecal bifidobacteria numbers was measured. Gibson et al. (1995) reported that total bifidobacteria increased significantly and their proportion of total anaerobes rose from 8% before to 20% after supplementation of a control diet with 15 g oligofructose/ d. Bartram et al. (1994) found no change in anaerobes during dietary supplementation of yogurt containing Streptococcus thermophilus, L. bulgaricus and B. longum, although concentration of fecal bifidobacteria increased significantly.

We observed a significant time by treatment interaction was inherent in the breath hydrogen test. Factors that may have affected changes in hydrogen excretion or results from the lack of reproducibility believed to be inherent in the breath hydrogen test. Factors that may change in hydrogen excretion include changes in colonic pH (Gibson et al. 1990, Perman et al. 1985), the rate of delivery of the substrate to the colon (Read et al. 1984), and colonic stirring and activity of hydrogen-consuming bacteria (Strochi and Levitt 1992). Additional influences on hydrogen excretion might include antibacterial treatment (Bjorneklett and Jenssen 1982, Gilat et al. 1978), mild exertion (Payne et al. 1983), and changes in ventilation as a result of tachypnea (Perman et al. 1985) or smoking (Tadese and Eastwood 1977). It is unlikely that these factors had an effect in this study, however, given the subject selection and breath hydrogen test criteria.

In addition to the colony count methodology described above, concurrent analysis of the exogenous fecal bifidobacteria was conducted in the laboratory by 16S rDNA RFLP. The presence of the exogenous bifidobacteria in the fecal samples confirmed that the ingested bifidobacteria remained viable in the colon. Although no significant quantitative change in the total fecal bifidobacteria population was observed during the consumption of bifidobacteria, the proportion of exogenous bifidobacteria in the colonic flora substantially increased and accounted for nearly half of the fecal bifidobacteria during this period. Ingestion of the exogenous bifidobacteria therefore appeared to exert a qualitative effect on the fecal bifidobacteria populations. The ingested bifidobacteria was absent in the feces of the subjects before the consumption of exogenous bifidobacteria. On d 4, $\sim 60\%$ of the total fecal bifidobacteria were the exogenous strain, and on d 8 and 12, the mean value was $\sim 70\%$ of the total bifidobacteria. On d 4 of the washout period, the mean fecal concentration decreased to $\sim 20\%$ of the total fecal bifidobacteria populations, and on d 8 of the washout period, the exogenous strain was not found in the fecal samples of any of the subjects. Calculations of the total number of exogenous bifidobacteria recovered after a period of daily consumption of $\sim 10^{10}$ CFU of the exogenous bifidobacteria suggest that $\geq 10^{10}$ CFU of the exogenous strain were present in a typical fecal sample. From these results, it appears that either equivalent numbers or slightly greater numbers of the exogenous bifidobacteria were recovered from the feces than were consumed. This suggests that the exogenous strain remained viable in its passage through the gastrointestinal tract and that the viable organisms managed to multiply. These findings are in contrast to those of Bouhnik et al. (1992), who recovered only 30% of the oral load in subjects’ feces.

Colonic hydrogen, a portion of which is excreted in breath, is a metabolite of fermentation in the colon. Breath excretion of hydrogen has been shown to correlate strongly ($r = 0.9$) with hydrogen concentration in the colonic lumen (Levitt 1969). The breath hydrogen response to a consistent dose of a nonabsorbable carbohydrate such as lactulose therefore provides an index of the fermentation activity of the flora in the right colon. In this study, the breath hydrogen test after a lactulose challenge was used to semiquantitatively monitor the effect of daily ingestion of $10^{10}$ cells bifidobacteria on breath hydrogen excretion. No significant difference was found in this study as measured by the breath hydrogen test before...
and immediately after an 11- to 14-d feeding period of the bacteria. Collectively, these findings appear to suggest that no less hydrogen gas was produced in the colon of the subjects after ingestion of bifidobacteria.

Several investigators have suggested that the breath hydrogen test does not generate reproducible results after administration of identical lactulose challenges. La Brooy et al. (1983) have denied the reproducibility of the lactulose hydrogen breath test in and between individuals. More recently, Gelissen and colleagues (1994) found considerable intraindividual variation between duplicate breath H₂ responses collected from subjects on two separate days in response to standard test meals. Corazza et al. (1993) also reported that respiratory H₂ excretion after lactulose ingestion is not consistent. Other investigators, however, have found the method to be acceptable when individual subjects serve as their own controls (Basilisco et al. 1985). Given that the breath hydrogen response represents the net hydrogen production in the colon rather than the absolute hydrogen production (Strochlic and Levitt 1992), the possibility that the activity of hydrogen-consuming bacterial populations has been altered cannot be excluded. The activity of these populations was not measured in this study.

Hertzler et al. (1997) recently found that the decrease in breath hydrogen observed with lactose feeding in a population of lactose-mal digesting individuals is due to decreased absolute hydrogen production rather than increased hydrogen consumption. The investigators postulated that daily consumption of lactose stimulated the proliferation of bacterial species such as bifidobacteria or other lactic acid bacteria that carry out non-hydrogen-producing, lactose-fermentation reactions. The concept of lactose adaptation does not appear likely in this study, however, given that all participants were identified as lactose digestors, and net hydrogen gas production did not significantly decrease after a period of daily milk consumption. The findings of Hertzler et al. (1997) raise an interesting point. During the study feeding periods, subjects were required to consume a minimum of 36 g lactose/d, whereas the habitual daily lactose intake of all subjects before the study was 24.7 ± 3.4 g (32.3 ± 5.7 g, men; 20.0 ± 3.6 g, women). These data suggest that subjects may have consumed considerably more lactose during the study than they consumed in their habitual diets.

In conclusion, it appears that daily consumption of viable bifidobacteria in skim milk did not significantly affect the numbers of fecal bifidobacteria or breath hydrogen excretion; however, our subjects showed high inter- and intraindividual variation. These data suggest that ingested bifidobacteria might not attenuate gas production in the colon.

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LITERATURE CITED


